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Extended Summaries 42nd Hungarian Plant Protection Days Meeting

The following are extended summaries based on papers presented at the 42nd Hungarian Plant Protection Days Meeting, organised by the Hungarian Agricultural Society, the Hungarian Academy of Sciences, the Hungarian Ministry of Agriculture and the Plant and Soil Protection and Soil Conservation Station of Budapest and held in Budapest, Hungary on 27/28 February, 1996. They are entirely the responsibility of the authors and do not necessarily reflect the views of the Editorial Board of Pesticide Science.

Effects of some Materials Extracted from *Ajuga reptans* var. *reptans* on *Aedes aegypti* and *Dysdercus cingulatus* Larvae

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Ajuga species (Labiatae), common name bugle, which have been used to treat sore throats, inflammation, infectious diseases, fevers, diabetes, hypertension and gastralgia in folk medicine, also produce allelochemicals which affect postembyronic development and reproduction in invertebrate herbivores.[1] Luteolin, apigenin and their glycosides; iridoids (harpagid, harpagidacetate); catechin-like compounds; caffeic, 4-caffeoylquinic and chlorogenic acids;[2] and a significant quantity of diterpenoid neo-clerodanes and phytoecdysteroids are formed as a result of secondary metabolism in these plants.[3] *Ajuga reptans* var. *reptans* L. produces the neo-clerodanes ajugareptansin and ajugareptanson and the phytoecdysteroids 20-hydroxyecdysone, polypodine B, cyasterone, norcyasterone, 2- and 3-acetyl-29-norcyasterone, ajugalactone, sengosterone and norsengosterone.[3,4]

A. reptans reptans has no specific insect pests, but is occasionally attacked by generalist herbivores.[5,6] Some extracts of *Ajuga* species from Japan were found to disrupt larval development of some insects[1] while a methanolic *A. reptans reptans* extract was reported to have deterrent and moulting inhibitor activities on the Mexican Bean Beetle *Epilachna varivestis* Muls.[7] and an ethanolic extract lowered the ecdysteroid level in cockroach (*Periplaneta americana* L.) larvae.[8] Food treated with acetonitrile extract of *A. reptans reptans* disturbed moulting during the larval development of the fleshfly *Neobellieria bullata* Parker, causing early head sclerotization during the wandering phase of the last larval stadium of *N. bullata* when used with Ohtaki-type wet synchronization.[9] Neo-clerodanes from *Ajuga spp.* had phagodeterrent activity on the cotton worm *Spodoptera littoralis* (Boisd.)[10] as well as on *P. americana*, resulting in a decrease in production and weight of oothecae.[9]

Our aim was to explore the insecticidal activity of the crude and fractionated methanolic extracts from different *A. reptans reptans* strains collected in different stages of plant growth.

Dried plant material (10 g) was ground and sonicated with methanol or acetonitrile (2 × 190 ml) for 5 min. In each case, the extracts were combined and solvent removed under vacuum and the residue was redissolved in methanol (10 ml) and stored at −50°C.

The solvent was removed from the methanolic extract in a stream of nitrogen and the residue was dissolved in distilled water and fractionated on a Sep-Pak C_{18} column. The eluant was methanol + water applied as a step-wise gradient from 5 + 95 (5M) to 100 + 0 (100M) by volume as indicated in Fig. 1; chloroform + methanol (1 + 1 by volume) was then used to elute non-polar substances (the MC fraction). The different allelochemicals were monitored by TLC on Kieselgel 60 F_{254} phase using dichloromethane + ethanol (85 + 15 by volume) or ethyl acetate + methanol + aqueous ammonia (85 + 10 + 5 by volume) as mobile phase, the compounds being visualised with vanillin and sulfuric acid.

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Fig. 1. Efficacy of fractions of *Ajuga reptans reptans f. macrofilia* leaf methanolic extract before flowering. W, M and MC represent water, methanol or methanol + chloroform fractions, respectively; IS implies material insoluble in water.

The fractions from the Sep-Pak C_{18} column[9] were examined by HPLC on a Lichrocart 125 × 4 mm column (packed with Lichrospher 100 RP-185 μm) using a flow rate of 1.2 ml min^{-1} at 55°C, the eluent being aqueous isopropanol (64 ml $litre^{-1}$) for 0–30 min, followed by a gradient to 144 ml isopropanol $litre^{-1}$ over 30–50 min and a final 50–70 min elution with this eluant.[4,11]

For biological assays, the solution in methanol from the Sep-Pak C_{18} column separation was evaporated to dryness in a stream of nitrogen, tap water (10 ml) was added and the mixture sonicated for 5 s to give a stock solution from which a range of dilutions was made to give samples corresponding to 0·05–1·0% of the dry weight of the plant sample from which the material originated. These dilutions were made either with water used for rearing[9] 15 mosquitos *Aedes aegypti* (L.) (Diptera, Culicidae) L_4 or with the drinking water from 25 Cotton Stainer Bugs *Dysdercus cingulatus* (F.) (Heteroptera, Pyrrhocoridae) L_2. The former is an Endopterygote species with 20-hydroxyecdysone as its moulting hormone and the latter is an Exopterygote species with makisterone A as its moulting hormone. The samples were monitored daily and the number of adults which emerged was recorded. Each sample was tested twice and probit analysis was used to calculate the LC_{50} values (the concentration at which 50% control of the target organism, compared with the untreated control, was achieved).

The phytoecdysteroid content of *A. reptans reptans* depends strongly on the strain, the plant part, the growth stage at collecting time[11] and the extraction procedure (Table 1).

There were considerable differences between the efficacies of the extracts from different *A. reptans reptans* strains. Leaves removed before flowering with the BS-1 strain from Germany produced 241 mg kg^{-1} phytoecdysteroids and had no effect on *D. cingulatus*, while the extract corresponding to 0·35% of the dry weight of BT-33 strain (*A. reptans reptans forma macrofolia*) produced 1567 mg kg^{-1} phytoecdysteroids and inhibited the treated larvae from developing into normal and fertile adults. The crude methanolic extracts, extracted from whole plants (i.e. leaf, root, and inflorescence) were always more effective on both test insects when sampled before flowering than after.

Based on these results, further work focused on the methanolic fractions of the leaves collected before flowering time with a Hungarian *A. reptans reptans* strain, referred to here as *forma macrofolia* (BT-33), the phytoecdysteroid content of which was *c.*1600 mg kg^{-1} when sampled in June. The major phytoecdysteroids

TABLE 1
Phytoecdysteroid Profile of *Ajuga reptans* var. *reptans*[a]

Phytoecdysteroids	*Acetonitrile extract BT-33*	*Methanolic extract*			
		BT-33	*BT-33*	*BT-33*	*BT-1*
	(*L*, *BF*)[b]	(*L*, *BF*)[b]	(*L*, *AF*)[b]	(*R*, *BF*)[b]	(*L*, *BF*)[b]
Polypodine B	62	110	59	64	15
Sengosterone	50	60	47	42	0
20-Hydroxyecdysone	420	707	362	122	8
Cyasterone	98	138	93	47	11
Ajugalactone	111	73	136	142	121
Other ecdysteroids	386	479	195	531	86
Total ecdysteroids	1127	1567	892	948	241

[a] Values are in mg kg^{-1} dry weight of plant material.
[b] AF and BF represent after and before flowering; L and R represent leaves and root, respectively; BS-1 is a strain originating from Germany and BT-33 the *forma macrofolia* strain from Hungary.

present were 20-hydroxyecdysone, cyasterone, polypodine B, ajugalactone and sengosterone, with 22-dehydro-12-hydroxycyasterone and 2-*O*-acetyl-20-hydroxyecdysone as minor components. Several very apolar phytoecdysteroids were also detected, but were not identified.

The fractions: 50 M–60 M, 100 M and the CM fraction (Fig. 1) were the most effective on *A. aegypti*. Most of the *A. aegypti* larvae/pupae died during L/P-moult or as a pharate imago. In the 55 M–60 M, 100 M and CM fractions, neo-clerodanes were the only known compounds identified. The phytoecdysteroids fractions, 35 M–45 M showed the strongest effect during the postembryonic development of *D. cingulatus* while the fractions 40 M–45 M decreased egg production. Insecticidal effects were also found in the iridoid-containing 20 M fraction (Fig. 1).

Our results suggest that: (a) neo-clerodanes (ajugareptansin and ajugareptanson) are responsible for the efficacy of *A. reptans reptans* extracts on *A. aegypti* larvae; (b) that *A. reptans reptans*-related neoclerodanes have juvenile hormone agonist-type activity during *A. aegypti* larval/pupal metamorphosis; (c) that *D. cingulatus* larvae, with the moulting hormone makisterone A, are more sensitive to exogenous phytoecdysteroids than are mosquito larvae; (d) that highly polar compounds have no activity on mosquito, but iridoids have activity on bug larvae; and (e) that highly non-polar compounds have no activity on bug larvae. Mosquito larvae could feed on dissolved compounds in the extracts while *D. cingulatus* tended to avoid ingestion.

Further studies in this area are in progress.

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